EPA BASE STUDY
STANDARD OPERATING PROCEDURE FOR SAMPLING AND CHARACTERIZATION OF VIABLE AND NON-VIABLE BIOAEROSOLS IN INDOOR AIR

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LIST OF ABBREVIATIONS AND ACRONYMS

BASE	Building Assessment Survey and Evaluation
IADCS	Indoor Air Data Collection Software
m^3	cubic meter
VOC	volatile organic compound

1.0 OBJECTIVE

The objective of the procedure is to collect a representative sample concentration of total airborne fungal spores (viable and non-viable) that may be present in indoor air and in the outdoor air supplied to the space tested. The most comprehensive method of sampling for the biological materials includes a combination of viable (Graseby-Andersen N6 Sampler) and total concentration methods (Burkard Volumetric Air Sampling Pump). The collected samples are subsequently cultured and/or microscopically counted to speciate and quantify the organisms collected.

Total fungal spores will be collected using a Burkard Volumetric Air Sampling Pump. This instrument utilizes a stop-cock grease-coated slide for spore deposition. The Burkard samples (bioaerosol: non-viable and viable species) will be conducted simultaneously with the Graseby-Andersen samples (bioaerosol: viable species). The bioaerosol samples are collected indoors (at Fixed Sites 1, 3, and 5, as defined in the *BASE Protocol*) and near the outdoor air intake for the study area.

1.1 THEORY/PRINCIPLE OF COLLECTION

The Volumetric Air Sampling Pump, manufactured by Burkard, Inc. (Rickmansworth, England) is designed for virtually 100 percent collection of all sizes of particles and spores to allow complete quantification of spores in a known volume of air. Since the collection media (a glass microslide) is not a colonizing medium, it serves the purpose of collecting both live and dead airborne spores.

The air stream enters the mouth of the air sampler and impacts a glass microslide that is lightly doused with stop-cock grease and is perpendicular to the air flow. Due to the high air velocity (approximately 3,000 feet per minute) at the impaction point and the rigorous turn the air must take around the slide, it can be assumed that all particles impact the slide and are retained in the grease. Analysis is performed using a microscope by physically counting and speciating fungal spores in the sample. To determine concentration, the spore count is divided by the volume of air collected.

2.0 GENERAL PROCEDURES

2.1 GENERAL SAMPLING CRITERIA AND REQUIREMENTS

Sampler (Burkard) Disinfection. Before each round of sampling, the sampler inlet on top of the pump is wiped with isopropyl alcohol-soaked cotton swabs or Kimwipes® and dried. This operation must be conducted in a location away from the BASE study space, as integrated sampling of volatile organic compounds (VOCs) is conducted in coincidence with bioaerosol sampling.

Collection Media. The samples are collected on glass slides. The glass slides are supplied and analyzed by Environmental Microbiology Laboratory in Escondido, California.

2.2 REQUIRED EQUIPMENT AND SUPPLIES

The equipment and supplies required to perform fungal spore sampling as part of the BASE study are as follows:

- Burkard Volumetric Air Sampling Pump and charger
- 3” x 1” (or 75 mm x 25 mm) glass slides
- stop-cock grease
- slide tray for containing and shipping the samples
- Isopropyl alcohol, cotton swabs or Kimwipes®
- Indoor Air Data Collection Software (IADCS) sample identification labels (affixed to glass slide, folding it around the edges)

2.3 SET-UP AND SAMPLING

The Burkard Sampler is charged for a minimum of 24 hours before sampling begins on Wednesday morning. For the afternoon (p.m.) sampling round, a second sample will serve as a replicate. Air is sampled for a time interval of four (4) minutes at a flow rate of approximately 14.5 liters/minute and for a total volume of approximately 58 liters. The sample duration was suggested by mycologists who have experience sampling the

indoor environment. The duration is thought to provide a reasonable sample volume to minimize the problems of sample overload and count underestimation.

Prior to sampling both indoors and outdoors, the top inlet of each pump is wiped with isopropyl alcohol using soaked cotton swabs of Kimwipes®. This cleaning is done at a location sufficiently removed from the BASE study space fixed sampling sites, since integrated sampling of volatile organic compounds (VOCs) is being conducted in parallel with bioaerosol sampling. Isopropyl alcohol will cause contamination of the VOC sample.

The samples from the Outdoor Site and Fixed Sites 1, 3, and 5 and the replicate sample are collected simultaneously with the Graseby-Andersen sampling. Each slide is carefully prepared by applying a small amount of stop-cock grease to a finger and spreading the grease thinly, covering only the central portion of the slide. It is important to apply only a very thin coating, as too much will make the spore count difficult. Align the red dots on the head of the Burkard Sampler and insert the slide, greased side up. To assure that the slide is properly inserted, use the Burkard key or the tip of a pencil to push the slide all the way into the sampler. Then rotate the head of the Burkard Sampler so that the red dots are 90° apart. Set the timer on the bottom of the Burkard Sampler for four (4) minutes, then switch the sampler on.

Record the *On* time and *Sample Location* on the field log sheet. After the sample terminates, use the key to remove the slide. Handle the slide by its edges only. Affix the IADCS-generated label on an end of the glass slide so that it folds around the edges. Record the IADCS sample ID on the field log sheet. Take the slide and hold it up to the light to see if there is a fine white line of collected debris, then place the slide back in the slide tray.

After the completion of sampling, the samples <u>are shipped on the same day of sampling by priority overnight delivery to the analyzing laboratory.</u>

2.4 SAMPLING SITE LOCATIONS

Samples are collected during the morning and during the afternoon of the day specified in the *BASE Protocol* at the following locations, provided the test space can accommodate this configuration.

- **Outdoor Site** (near the outdoor air intake for the study area): four-minute sample
- **Fixed Site 1**: four-minute sample
- **Fixed Site 3**: four-minute sample
- **Fixed Site 5**: four-minute sample
- **Duplicate Site** (indoors): four-minute sample

3.0 CALIBRATIONS AND QUALITY CONTROL

3.1 CALIBRATION

The Burkard Volumetric Air Sampling Pumps are calibrated annually. Because the pumping mechanism is a high flow, low pressure centrifugal fan, care must be taken during calibration to ensure that the flow measurement device does not cause any flow obstruction, which would lead to false low calibration results. Hence, a short ¾ inch diameter PVC tube is used at the mouth of the sampler in conjunction with a calibrated hot wire anemometer (TSI VelociCalc® Plus or equivalent). Flow rate, in liters per minute, is calculated using a traverse of the ¾ inch tube face velocity and multiplying that result by the cross-sectional area of the tube. Due to the inherently stable nature of the pumping device and the fact that the flow rate of the unit cannot be manually adjusted, field calibrations are not required.

3.2 QUALITY CONTROL SAMPLES

Due to the unpredictable nature of spore concentrations in outdoor environments, there will be only one replicate sample collected per building at the indoor duplicate site. Generally, only one Burkard sampler is available to each field team; therefore, it is not possible to run a duplicate in coincidence with its primary sample. Instead a replicate sample is taken immediately after the completion of the primary sample.

Because the analysis method allows for each sample to serve as a blank, no field blank will be taken.

4.0 SHIPPING PROCEDURES

Immediately after sampling, the samples are shipped to the analyzing laboratory for receipt at the laboratory within 24 hours of collection. Arrangements must be made prior to each sample collection sequence to ensure timely reception and processing of the samples at the laboratory.

After sampling, the slides are placed back into the slide tray. To prevent possible damage in shipment, a folded Kimwipe® may be placed over the slides before closing the tray.

5.0 SAMPLE ANALYSIS

The collected samples must be analyzed under the supervision of a person with "demonstrated experience in the handling and analysis of environmental isolates." The following laboratory has been chosen to perform analyses:

Environmental Microbiology Laboratories
11746 Alps Way
Escondido, CA 92026
Attn: Ms. Janet Gallup

The data will be reported as follows:

- **genera spores/cubic meter (m^3)**
- **total raw spore count**
- **total spores/m^3**

APPENDIX A

SAMPLE SPREADSHEET
FOR ANALYTICAL RESULTS

GENERAL INFORMATION	DATA CODES	SAMPLING INSTRUMENT	
BUILDING EVENT CODE:	-95 - sample voided by lab	Burkard spore trap	
# SAMPLES IN THIS SET:	-96 - sample not analyzed	(Non viable methodology)	
Sampling date:	-97 - < minimum detection limit	LAB DETECTION LIMITS (THIS SAMPLE SET)	
Initials:	-98 - < minimum quantitation limit	3 minute sample 33 spores/m3 detection limit	5 minute sample 20 spores/m3 detection limit
	-99 - < highest calibration standard		

MOLD SPORE & POLLEN REPORT

Sample ID	Sample volume (m3	Alternaria pores/m	Arthrinium pores/m	Ascospores* spores/m3	Aurobasidium pullulans (spores/m3	Basidiospores* (spores/m3)	Botrytis pores/m	Chaetomium spores/m3	Cladosporium (spores/m3)	Curvularia pores/m	Drechslera / Bipolaris group (spores/m3)	Epicoccum pores/m	Fusarium pores/m	Myrothecium spores/m3	Nigrospora pores/m	Other colorless pores/m

† The spores of *Aspergillus* and *Penicillium* (and others such as *Acremonium, Paecilomyces*) are small and round with very few distinguishing characteristics. They cannot be differentiated by non viable sampling methods. Also, some species with very small spores are easily missed, and may

* Most of these spore types are not seen with viable sampling methods (Anderson sampling), although some may appear as sterile mycelia. Most of the basidiospores are 'mushroom' spores while the rusts and smuts are plant pathogens.

Comments:

Penicillium / Aspergillus types† (spores/m3)	Pithomyces pores/m	Rusts* pores/m	Smuts*, Periconia, Myxomycetes* (spores/m3)	Stachybotrys spores/m3	Stemphylium (spores/m3	Torula herbarum pores/m	Ulocladium pores/m	Unknown pores/m	Zygomycetes (possible) spores/m3)	Background debris	Total raw spore count	Total spores/m3	Comments